molecular probes®
by life technologies™

Tyramide Signal Amplification Kits

Table 1 Contents and storage

Material*	Amount	Storage	Stability
Labeled tyramide (Component A)†	1 vial	• ≤–20°C • Desiccate • Protect from light	When stored as directed the product is stable for at least 1 year.
Dimethylsulfoxide (DMSO) (Component B)	200 mL		
HRP-conjugated secondary antibody or streptavidin (Component C)	20 µg or 100 µg		
Blocking reagent (Component D)	3 g of Bovine Serum Albumin (BSA)		
Amplification buffer (Component E)	25 mL (contains thimerosal at 0.02%)		
Hydrogen peroxide (H_2O_2) (Component F)	200 µL of a 30% of a stabilized solution		

* Tyramide stand-alone reagent kits only provide Component A and Component E. Other reagents will have to be provided and their concentration optimized by the user. We strongly recommend the first time user to purchase and use the whole kit rather than the stand-alone reagent kits. Anhydrous DMSO for dissolving the stand-alone tyramides can be purchased separately from Life Technologies (Cat. no. D12345)

† Component A is difficult to see and the vial may appear to be empty.

Number of assays: Each kit provides sufficient materials to stain 50–150 slides.

Approximate fluorescence excitation and emission maxima: See Table 2, page 3.

Introduction

Tyramide Signal Amplification (TSA™) is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase (HRP) to generate high-density labeling of a target protein or nucleic acid sequence *in situ*.[1–5] TSA™ labeling is a combination of three elementary processes (Figure 1, page 2):

1. Binding of a probe to the target via immunoaffinity (proteins) or hybridization (nucleic acids) followed by secondary detection of the probe with an HRP-labeled antibody or streptavidin conjugate.

2. Activation of multiple copies of a dye- or hapten-labled tyramide derivative by HRP.

3. Covalent coupling of the resulting highly reactive, short-lived tyramide radicals to nucleophilic residues in the vicinity of the HRP–target interaction site (Figure 2, page 2), resulting in minimal diffusion-related loss of signal localization.

For Research Use Only. Not for use in diagnostic procedures.

The signal amplification conferred by the turnover of multiple tyramide substrates per peroxidase label translates to practical benefits, namely ultrasensitive detection of low-abundance targets and the use of smaller amounts of antibodies and hybridization probes. In immunohistochemical applications, sensitivity enhancements derived from TSA™ method allow primary antibody dilutions to be increased to reduce nonspecific background signals,[6] and can overcome weak immunolabeling caused by suboptimal fixation procedures[7] or low levels of target expression.[8] The lower detection threshold of TSA™ method compared to fluorescent secondary antibodies also allows detection of two targets with primary antibodies raised in the same host species but without substantial crosstalk between the signals.[7,9]

The increased sensitivity afforded by the TSA™ method can be critically important for detection of short oligonucleotide probes and low-abundance mRNAs by fluorescence *in situ* hybridization (FISH).[4,10] Optimal probe concentrations are typically 2–10 fold lower for TSA™-detected FISH than for conventional immunocytochemical detection procedures.[11]

Higher levels of signal amplification can also be achieved by detection of Oregon Green® 488 tyramide with anti-fluorescein/Oregon Green® antibody conjugates labeled with horseradish peroxidase (for second-stage TSA™ signal generation) or with fluorescent dyes. As with other detection systems, TSA™ method allows identification of multiple targets by simultaneously hybridized probes; however, signal development using tyramides coupled to different fluorophores must be carried out sequentially with a peroxidase inactivation step between each TSA™ reaction, to prevent crosstalk.[11]

Figure 1 Schematic representation of TSA™ detection applied to immunolabeling of an antigen.

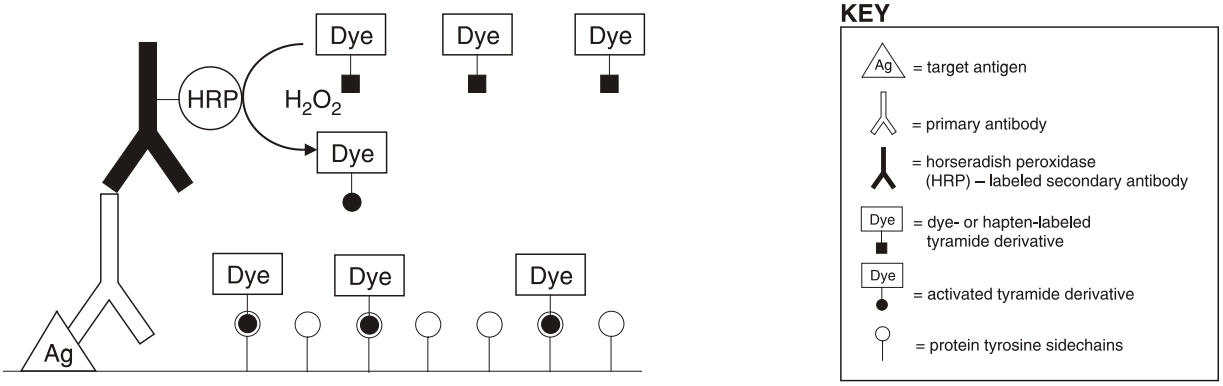
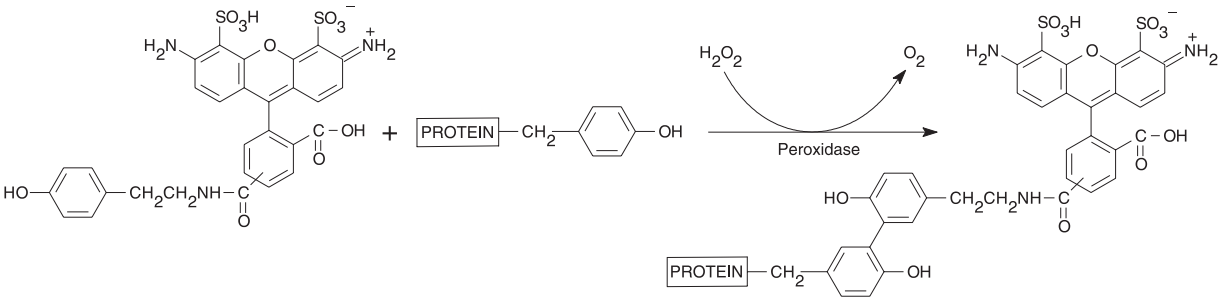


Figure 2 HRP-mediated coupling of Alexa Fluor® 488 tyramide to protein tyrosine residues.



Materials

Life Technologies offers a full line of TSA™ kits (Table 2, below) for detection strategies to match the user's primary detection reagent and available instrumentation. TSA™ kits with goat anti–mouse IgG–HRP conjugate are for detecting mouse primary antibodies; kits with goat anti–rabbit IgG–HRP conjugate are for detecting rabbit primary antibodies; and kits with streptavidin–HRP conjugate are for detecting biotinylated targets. One of the three different HRP conjugates is provided in combination with a labeled tyramide. Fluorescent labels include our superior Alexa Fluor® dyes and the Oregon Green® 488 dye. Oregon Green® 488 dye provides not only a green fluorescent signal, but also can serve as a hapten in conjunction with our antifluorescein/Oregon Green® antibodies (available separately) for an additional level of signal amplification. Biotin-XX–tyramide, which has a 14-atom linker for increased accessibility, is used for indirect labeling strategies involving dye-labeled or enzyme-labeled streptavidin (available separately).

Table 2 TSA™ detection kits.

			Horseradish peroxidase conjugate		
Labeled tyramide	Ex/Em*	Stand-alone†	Anti–mouse IgG (host = goat)	Anti–rabbit IgG (host = goat)	Streptavidin
Alexa Fluor® 350	347/442		T20917	T20927	T20937
Alexa Fluor® 488	495/519	T20948	T20912	T20922	T20932
Oregon Green® 488	496/524				T20939
Alexa Fluor® 546	556/573		T20913	T20923	T20933
Alexa Fluor® 555	555/565		T30953	T30954	T30955
Alexa Fluor® 568	579/604	T20949	T20914	T20924	T20934
Alexa Fluor® 594	591/617	T20950	T20915	T20925	T20935
Alexa Fluor® 647	650/668	T20951	T20916	T20926	T20936
Biotin-XX	NA	T20947	T20911	T20921	T20931

* Ex/Em = Fluorescence excitation/emission maxima, in nm. NA = not applicable.

† Tyramide stand-alone reagents kits only provide labeled tyramide (Component A) and amplification buffer (Component E). Other reagents will have to be provided and their concentration optimized by the user. Anhydrous DMSO for dissolving the stand-alone tyramides can be purchased separately from Life Technologies (Cat. no. D12345). We strongly recommend the first time user to purchase and use the whole kit rather than the stand-alone reagent kits.

Reagent Preparation

1.1 Prepare phosphate-buffered saline (PBS) (not provided)according to standard laboratory protocols.

1.2 Prepare tyramide stock solution by dissolving the solid material provided (Component A) in 150 µL of DMSO (Component B). Invert the vial several times to dissolve any tyramide coating the sides of the vial. Store unused portions of this stock solution in small aliquots at ≤–20°C, desiccated and protected from light.

Note: Anhydrous DMSO for dissolving the stand-alone tyramides can be purchased separately from Life Technologies (Cat. no. D12345).

1.3 Prepare a 1% (10 mg/mL) solution of blocking reagent (BSA) in PBS. We recommend preparing only as much as is needed for immediate use. However, unused solution can be stored frozen at ≤–20°C for 1 month if necessary.

1.4 Prepare the HRP conjugate stock solution by reconstituting the material provided in 200 µL of PBS. This solution may be stored at 2–8°C for up to 3 months if required. Optionally, add 0.02% thimerosal as a preservative. Note that sodium azide must NOT be used for this purpose.

1.5 Prepare amplification buffer/0.0015% H_2O_2 by adding 30% hydrogen peroxide (Component F) to amplification buffer (Component E) to obtain a final concentration of 0.0015% H_2O_2. For example, add 1 µL of 30% H_2O_2 to 200 µL of amplification buffer and then add 1 µL of this intermediate dilution (0.15% H_2O_2) to a further 100 µL of amplification buffer. We recommend preparing 100 µL of this working solution per sample.

1.6 Prepare peroxidase quenching buffer (not provided, use is optional): PBS + 1–3% H_2O_2.

Cell Fixation

Follow customary cell or tissue fixation procedures. The following procedure is provided as a guide only.

2.1 Rinse the cells or tissue free of culture medium with PBS that has been warmed to 37°C.

2.2 Fix the cells or tissue with 3.7% formaldehyde, or paraformaldehyde, in PBS at room temperature for 20 minutes. The fixative solution may also be warmed to 37°C if cells are sensitive to temperature shock.

2.3 Rinse the cells or tissue with PBS.

2.4 Permeabilize the cells with 0.1–0.2% Triton® X-100 solution for 5–10 minutes at room temperature, or with acetone at ≤–20°C for 10 minutes.

2.5 Rinse the cells or tissue with PBS.

Peroxidase Labeling

3.1 If necessary, quench endogenous peroxidase activity by incubating in peroxidase quenching buffer for 60 minutes at room temperature.

3.2 Incubate the specimen with 1% blocking reagent for 60 minutes at room temperature or 37°C.

3.3 Label the cells or tissue with primary antibody diluted in 1% blocking reagent for 60 minutes at room temperature (see **Note A**, page 7).

3.4 Rinse the cells or tissue three times with PBS.

3.5 Prepare a working solution of the HRP conjugate by diluting the stock solution (prepared in step 1.4) 1:100 in 1% blocking solution (see **Note B**, page 7). A 100-µL volume of this working solution is sufficient to cover a standard 18 × 18 mm coverslip.

3.6 Apply 100 µL of the HRP conjugate working solution to the cells or tissue and incubate for 30–60 minutes at room temperature.

3.7 Rinse the cells or tissue three times with PBS.

Tyramide Labeling

4.1 Prepare a tyramide working solution by diluting the tyramide stock solution 1:100 (see **Note C**, page 7) in amplification buffer/0.0015% H_2O_2 (prepared in step 1.5) just prior to labeling. Prepare 100 µL of working solution per specimen. This quantity is sufficient to cover a standard 18 × 18 mm coverslip.

4.2 Apply 100 µL of the tyramide working solution to the cells or tissue and inclubate for 5–10 minutes at room temperature.

4.3 Rinse the cells or tissue three times with PBS. Fluorescence detection of deposited biotin-XX tyramide requires the application of a fluorophore-labeled streptavidin conjugate (not provided).

4.4 Mount the specimen (see **Note D**, page 7) and examine by fluorescence microscopy or other appropriate imaging method.

Fluorescence Detection

Approximate excitation and emission maxima for visualization of TSA™ labeling by fluorescence microscopy are listed in Table 2, page 3. Use the appropriate filter set to visualize the TSA™ label.

Signal Characteristics

Background fluorescence may originate from several sources. Due to the amplified detection inherent in the TSA™ process, background staining that was previously undetectable may become more prominent. Diffuse background fluorescence in the cytosol or surrounding tissue may be due to nonspecific binding of antibodies or tyramides. Perform the necessary controls to identify the source of the background. Speckled staining patterns, particularly when they are cell-associated, may indicate nonspecific binding of either primary or secondary antibodies. Perform appropriate controls to identify the source. Intense, diffuse staining or punctate localized staining may be caused by nonspecific reaction of the tyramide with endogenous peroxidases. Incubation with the peroxidase quenching buffer (step 3.1) may alleviate this problem.

In some cases when the target is relatively abundant, the labeling reaction is so intense that diffusion of the tyramide reaction product can result in loss of signal localization. In such cases, improved resolution may be obtainable by attenuating the staining reaction. This can be done in several ways—reducing the concentration of tyramide or peroxidase conjugate applied to the specimen or shortening the reaction time.

The use of streptavidin–HRP as the secondary detection reagent can result in mitochondrial staining of the endogenous biotinylated mitochondrial proteins.[13] Effective blocking strategies have been developed to minimize this artifact, and Life Technologies offers the Endogenous Biotin-Blocking Kit (Cat. no. E21390) for this purpose.

Fluorescence In Situ Hybridization

The following protocol has been developed and used for *in situ* hybridization of biotinylated DNA probes to chromosome spreads, followed by TSA™ detection with streptavidin–HRP and dye–labeled tyramides.

Reagent Preparation

5.1 Prepare phosphate-buffered saline (PBS) (not provided) according to standard laboratory protocols.

5.2 Prepare tyramide stock solution by dissolving solid material provided (Component A) in 150 µL of DMSO (Component B). Store unused portions of this stock solution in small aliquots at ≤–20°C, desiccated and protected from light.

Note: Anhydrous DMSO for dissolving the stand-alone tyramides can be purchased separately from Life Technologies (Cat. no. D12345).

5.3 Prepare a 1% (10 mg/mL) solution of blocking reagent in PBS. We recommend preparing only as much as is needed for immediate use. However, unused solution can be stored frozen at ≤–20°C for 1 month if necessary.

5.4 Prepare a streptavidin–HRP stock solution by reconstituting the material provided in 200 µL of PBS. This solution may be stored at 2–8°C for up to 3 months if required. Optionally, add 0.02% thimerosal as a preservative. Note that sodium azide must NOT be used for this purpose.

5.5 Prepare amplification buffer/0.0015% H_2O_2 by adding 30% hydrogen peroxide (Component F) to amplification buffer (Component E) to obtain a final concentration of 0.0015% H_2O_2. For example, add 1 µL of 30% H_2O_2 to 200 µL of amplification buffer and then add 1 µL of this intermediate dilution (0.15% H_2O_2) to a further 100 µL of amplification buffer. We recommend preparing 100 µL of this working solution per sample.

Staining and Detection

6.1 Hybridize biotinylated DNA probes to chromosome spreads using standard procedures. Wash the slide and transfer it to a Coplin jar containing 2X SSC (sodium chloride/sodium citrate), pH 7.0.

6.2 Block the slide with 100 µL of 1% blocking reagent in PBS and cover it with a plastic coverslip. Incubate the slide in a humidified box at room temperature for 30 minutes.

6.3 Prepare a working solution of streptavidin–HRP by diluting the stock solution (prepared in step 5.4) 1:100 in blocking reagent.

6.4 Remove the plastic coverslip, add 100 µL of the streptavidin–HRP working solution to the slide and cover with a plastic coverslip in the humidified box at room temperature for 30 minutes.

6.5 Remove the coverslip and wash the slide three times by immersion in PBS wash buffer for 5 minutes each time at 37°C.

6.6 Prepare a tyramide working solution by diluting the tyramide stock solution 1:100 in amplification buffer/0.0015% H_2O_2 (prepared in step 5.5).

6.7 Add 100 µL of the tyramide working solution to the specimen, cover with a plastic coverslip, and incubate for 5–10 minutes at room temperature.

6.8 Repeat the washing procedure described in step 6.5.

6.9 Counterstain the slide with 15 nM DAPI for 1 minute. Rinse the slide with PBS after counterstaining.

6.10 Mount the slide in PBS/50% glycerol medium (see **Note D**, below).

6.11 Examine the specimen by fluorescence microscopy using optical filters appropriate for DAPI and the fluorescent tyramide of choice (see Table 2, page 3, for excitation and emission maxima).

Notes

[A] Sensitivity enhancements derived from TSA™ method typically allow primary antibody dilutions to be increased 5- to 50-fold from the levels used in conventional immunocytochemical protocols.[14] However the extent of dilution possible in any particular experiment will depend on the cell or tissue type, fixation methods and antibody characteristics.

[B] Dilutions of the HRP conjugate stock solution between 1:50 and 1:200 can generally be used, depending on the abundance of the target primary antibody.

[C] Dilutions of the tyramide stock solution between 1:50 and 1:200 can be generally be used to produce variations of staining intensity without compromising target resolution or background signal levels.

[D] PBS/50% glycerol medium is suitable for mounting specimens for immediate examination. For longer-term storage and antifade protection, we recommend our ProLong® Gold antifade reagent/mounting medium.[9]

References

1. J Immunol Methods 125, 279 (1989); **2.** Cytometry 23, 48 (1996); **3.** J Histochem Cytochem 45, 375 (1997); **4.** J Histochem Cytochem 47, 281 (1999); **5.** US Patent 5,196,306; **6.** J Histochem Cytochem 40, 1457 (1992); **7.** J Histochem Cytochem 44, 1353 (1996); **8.** J Histochem Cytochem 45, 315 (1997); **9.** Methods 18, 459 (1999); **10.** J Histochem Cytochem 47, 431 (1999); **11.** Current Protocols in Cytometry, J.P Robinson, Ed., pp 8.9.1–8.9.16, John Wiley & Sons, (2000); **12.** J Histochem Cytochem 48, 1593 (2000); **13.** J Histochem Cytochem 45, 1053 (1997); **14.** J Histochem Cytochem 45, 1455 (1997).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
T20911	TSA™ Kit #1 *with HRP–goat anti-mouse IgG and biotin-XX tyramide* *50–150 slides*	1 kit
T20912	TSA™ Kit #2 *with HRP–goat anti-mouse IgG and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20913	TSA™ Kit #3 *with HRP–goat anti-mouse IgG and Alexa Fluor® 546 tyramide* *50–150 slides*	1 kit
T20914	TSA™ Kit #4 *with HRP–goat anti-mouse IgG and Alexa Fluor® 568 tyramide* *50–150 slides*	1 kit
T20915	TSA™ Kit #5 *with HRP–goat anti-mouse IgG and Alexa Fluor® 594 tyramide* *50–150 slides*	1 kit
T20916	TSA™ Kit #6 *with HRP–goat anti-mouse IgG and Alexa Fluor® 647 tyramide* *50–150 slides*	1 kit
T20917	TSA™ Kit #7 *with HRP–goat anti-mouse IgG and Alexa Fluor® 350 tyramide* *50–150 slides*	1 kit
T20921	TSA™ Kit #11 *with HRP–goat anti-rabbit IgG and biotin-XX tyramide* *50–150 slides*	1 kit
T20922	TSA™ Kit #12 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20923	TSA™ Kit #13 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 546 tyramide* *50–150 slides*	1 kit
T20924	TSA™ Kit #14 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 568 tyramide* *50–150 slides*	1 kit
T20925	TSA™ Kit #15 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 594 tyramide* *50–150 slides*	1 kit
T20926	TSA™ Kit #16 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 647 tyramide* *50–150 slides*	1 kit
T20927	TSA™ Kit #17 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 350 tyramide* *50–150 slides*	1 kit
T20931	TSA™ Kit #21 *with HRP–streptavidin and biotin-XX tyramide* *50–150 slides*	1 kit
T20932	TSA™ Kit #22 *with HRP–streptavidin and Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20933	TSA™ Kit #23 *with HRP–streptavidin and Alexa Fluor® 546 tyramide* *50–150 slides*	1 kit
T20934	TSA™ Kit #24 *with HRP–streptavidin and Alexa Fluor® 568 tyramide* *50–150 slides*	1 kit
T20935	TSA™ Kit #25 *with HRP–streptavidin and Alexa Fluor® 594 tyramide* *50–150 slides*	1 kit
T20936	TSA™ Kit #26 *with HRP–streptavidin and Alexa Fluor® 647 tyramide* *50–150 slides*	1 kit
T20937	TSA™ Kit #27 *with HRP–streptavidin and Alexa Fluor® 350 tyramide* *50–150 slides*	1 kit
T20939	TSA™ Kit #29 *with HRP–streptavidin and Oregon Green® 488 tyramide* *50–150 slides*	1 kit
T20947	TSA™, biotin-XX tyramide* *50–150 slides*	1 kit
T20948	TSA™, Alexa Fluor® 488 tyramide* *50–150 slides*	1 kit
T20949	TSA™, Alexa Fluor® 568 tyramide* *50–150 slides*	1 kit
T20950	TSA™, Alexa Fluor® 594 tyramide* *50–150 slides*	1 kit
T20951	TSA™, Alexa Fluor® 647 tyramide* *50–150 slides*	1 kit
T30953	TSA™ Kit #40 *with HRP–goat anti-mouse IgG and Alexa Fluor® 555 tyramide* *50–150 slides*	1 kit
T30954	TSA™ Kit #41 *with HRP–goat anti-rabbit IgG and Alexa Fluor® 555 tyramide* *50–150 slides*	1 kit
T30955	TSA™ Kit #42 *with HRP–streptavidin and Alexa Fluor® 555 tyramide* *50–150 slides*	1 kit

Related Products

D12345	DMSO (dimethylsulfoxide), anhydrous	10 × 3 mL
P10144	ProLong® Gold antifade reagent	2 mL
P36930	ProLong® Gold antifade reagent	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 × 2 mL

Purchaser Notification

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Additional international offices are listed at
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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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